Appl. No. 09/442,111
Amdt. dated April 12, 2007
Amendment under 37 CFR 1.116 Expedited Procedure
Examining Group 1652

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-52. (Cancelled)

53. (Currently amended) A method of producing a product saccharide, wherein the product saccharide is an oligosaccharide or glycolipid, the method comprising

i) permeabilizing a microorganism or plant cell wherein the cell comprises:

a) a heterologous accessory enzyme for forming a nucleotide sugar; and

b) a heterologous glycosyltransferase;

~~i)~~ ii) contacting ~~a~~ the permeabilized microorganism or plant cell with an exogenous acceptor saccharide, wherein the ~~cell comprises:~~

~~a) a heterologous accessory enzyme for forming a nucleotide sugar; and~~

~~b)~~ a heterologous glycosyltransferase ~~which~~ catalyzes the transfer of a sugar from the nucleotide sugar to the acceptor saccharide to produce the product saccharide; and

~~ii)~~ iii) allowing formation of the nucleotide sugar and transfer of a sugar from the nucleotide sugar to the acceptor saccharide to form the product saccharide.

54. (Cancelled)

55. (Previously presented) The method of claim 53, wherein the heterologous glycosyltransferase is endogenous to the cell and is produced by the cell at an elevated level compared to a wild-type cell.

56. (Original) The method of claim 53, wherein the product saccharide is produced at a concentration of at least about 1 mM.

57. (Currently amended) The method of claim 53, wherein the cell is permeabilized using 1% Xylene.

58-59. (Cancelled)

60. (Previously presented) The method of claim 53, wherein the heterologous accessory enzyme is one or more of:

a GDP-mannose dehydratase, a GDP-4-keto-6-deoxy-D-mannose 3,5-epimcrase, and a GDP-4-keto-6-deoxy-L-glucose 4-reductase;

a UDP-galactose 4' epimerase;

a UDP-GalNAc 4' epimerase;

a CMP-sialic acid synthetase;

a pyrophosphorylase selected from the group consisting of a UDP-Glc pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a GDP-mannose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase; a kinase selected from the group consisting of myokinase, pyruvate kinase, acetyl kinase, creatine kinase, UDP-Glc-dehydrogenase; and

pyruvate decarboxylase.

61. (Previously presented) The method of claim 53, wherein the heterologous accessory enzyme and the glycosyltransferase are expressed as a fusion protein.

62. (Original) The method of claim 61, wherein the fusion protein comprises a CMP-sialic acid synthetase activity and a sialyltransferase activity.

63. (Original) The method of claim 61, wherein the fusion protein comprises a galactosyltransferase activity and a UDP-Gal 4' epimerase activity.

64. (Original) The method of claim 61, wherein the fusion protein comprises a GalNAc transferase activity and a UDP-GlcNAc 4' epimerase activity.

65. (Original) The method of claim 53, wherein the nucleotide sugar is GDP-fucose and the glycosyltransferase is a fucosyltransferase.

66. (Original) The method of claim 53, wherein the cell forms the nucleotide sugar at an elevated level compared to a wild-type cell.

67. (Original) The method of claim 66, wherein the elevated level of nucleotide sugar results from a deficiency in the ability of the cell to incorporate the nucleotide sugar into a polysaccharide normally produced by the cell.

68. (Original) The method of claim 67, wherein the deficiency is due to a reduced level of a polysaccharide glycosyltransferase activity.

69. (Original) The method of claim 53, wherein the cell/nucleotide sugar are selected from the group consisting of:

Azotobacter vinelandii/GDP-Man;
Pseudomonas sp./UDP-Glc and GDP-Man;
Rhizobium sp./UDP-Glc, UDP-Gal, GDP-Man;
Erwinia sp./UDP-Gal, UDP-Glc;
Escherichia sp./UDP-GlcNAc, UDP-Gal, CMP-NeuAc, GDP-Fuc;
Klebsiella sp./UDP-Gal, UDP-GlcNAc, UDP-Glc, UDP-GlcNAc;
Hansenula jadinii/ GDP-Man, GDP-Fuc;
Candida famata/UDP-Glc, UDP-Gal, UDP-GlcNAc;
Saccharomyces cerevisiae/UDP-Glc, UDP-Gal, GDP-Man, GDP-GlcNAc; and
X. campesti/UDP-Glc, GDP-Man.

70. (Original) The method of claim 53, wherein the cell is *Azotobacter vinelandii*, the nucleotide sugar is GDP-mannose, the acceptor saccharide is lactose, the glycosyltransferase is mannosyl transferase, and the product saccharide is mannosyl lactose.

71. (Currently amended) The method of claim 53, wherein the cell is *E. coli*, the nucleotide sugar is CMP-sialic acid, the acceptor saccharide is lactose, the glycosyltransferase is a sialyltransferase, the heterologous accessory enzyme is CMP-sialic acid synthetase, and the product saccharide is sialyllactose.

72. (Previously presented) The method of claim 53, wherein the glycosyltransferase consists essentially of a catalytic domain of the glycosyltransferase.

73. (Previously presented) The method of claim 53, further comprising the step of detecting the product saccharide.

74. (Previously presented) The method of claim 53, further comprising the step of isolating the product saccharide.